

Call repertoire of an Australian treefrog, *Litoria adelaidensis* (Anura, Hylidae)

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Abstract

Litoria adelaidensis (Anura: Hylidae) has a call repertoire that includes two structurally different call types. Males produced three recognizable pulsed calls (call types 1 to 3) and one unpulsed call (call type 4). All call types have two distinct frequency peaks. Call type 1 was produced most often, suggesting that this signal functions to attract females. Call type 4 was rarely produced and may be associated with male-male interactions, as males in close proximity to each other were observed making this call. The purposes of call types 2 and 3 are unknown. The different calls may be part of a graded response incorporating both male-male interaction and female attraction.

Key words: frog, vocal repertoire, advertisement call, acoustic communication, Hylidae, *Litoria adelaidensis*

Introduction

Organisms that emit acoustic signals often have a repertoire of calls, the extent of which reflects the strength and complexity of both sexual and natural selection (Narins *et al.* 2000). Female frogs typically use the male call to locate and choose between potential mates and may therefore impose strong selection on call structure (Gerhardt 1994). Male frogs can use acoustic signals in response to physical and/or acoustic intrusions by other males (Narins *et al.* 2000) and may use conspecific calls to determine the proximity (Brenowitz *et al.* 1984), size (Davies & Halliday 1978; Wagner 1992) and possibly condition of rivals (Halliday & Tejedo, 1995). Although many species use a single call for both functions (*e.g.* Littlejohn 1977; Wells 1977; Halliday & Tejedo 1995), a number have developed more complex signal repertoires (*e.g.* Given 1987; Narins *et al.* 2000) in which different calls serve different functions (*e.g.* Robertson 1984; Littlejohn & Harrison 1985).

Male frogs can produce acoustic signals that differ in both temporal and spectral structure (*e.g.* Ovaska & Calbeck 1997; Jehle & Arak 1998). *Hyla regilla* provides an excellent example, as it produces three distinct signals with similar spectral characteristics but different temporal properties (Brenowitz *et al.* 2001). A diphasic "advertisement call" is emitted commonly throughout the night and appears to attract females and to influence male spacing (Whitney & Krebs 1975a,b). A monophasic "advertisement call" is emitted at a high rate when females approach and is produced until the male has entered into amplexus with the female, but its exact function remains unclear (Brenowitz *et al.* 2001). *Hyla regilla* also produce an "encounter call" that is believed to be an aggressive signal that is important in the establishment of spacing between calling males

(Brenowitz *et al.* 2001). Males will switch to the encounter call after hearing the signal of an "intruding male".

Not only do male frogs often produce several different signals, but the signals may vary in a graded fashion from one extreme to another or from one call to another (Gerhardt & Huber 2002). Graded signals are typically produced in response to external stimuli such as conspecific male calls and/or physical intrusions or the approach of a potential mate. The advertisement call of *Rana nicobariensis*, for example, varies considerably in duration (approximately 20 fold) and the duration of the call is inversely related to the distance between the focal calling male and his nearest calling neighbour (Jehle & Arak 1998). Further, some species produce compound calls that incorporate both advertisement and aggressive signals (*cf.* Jehle & Arak 1998). Accordingly, the degree of within and between species variation in the acoustic signals of frogs contrasts with the view of early researchers that the calls of anurans were highly stereotyped and discrete (*e.g.* Blair 1958; Bogert 1960). The acoustic signals made by frogs show great variability which can be generated by natural, inter-sexual and intra-sexual selection (Gerhardt & Huber 2002).

A first step in studying anuran vocalizations is to record and quantify the types of calls that are produced by a species and to associate them with possible functions. Although there are extensive data on calls of Australian myobatrachid frogs (*e.g.* Littlejohn 1959; Littlejohn & Main 1959; Roberts & Wardell-Johnson 1995; Roberts 1997) and investigations of their function (*e.g.* Robertson 1984; Littlejohn & Harrison 1985; Gerhardt *et al.* 2000), comparatively few studies have investigated the call structure or call function for Australian hylid species. Here we quantify the acoustic signals of a south-western Australian frog, the slender treefrog, *Litoria adelaidensis*. The vocal repertoire of this species has not been described, but anecdotal evidence suggests that *L. adelaidensis* emits several different calls.

Materials and methods

Terminology

Anurans typically produce an advertisement call that is used to attract females and in some species to also mediate male-male interactions (Littlejohn 1977). In addition to the advertisement call, repertoires often include separate signals that function in male-male interactions such as an aggressive call, encounter call, or rivalry call (hereafter referred to as aggressive calls; Gerhardt & Huber 2002). Frogs may also produce short-range courtship signals (Gerhardt & Huber 2002) and/or distress and release calls (Bogert 1960). The advertisement call is usually repeated quasi-periodically for many hours per night during the breeding period (Narins *et al.* 2000). The aggressive calls are typically emitted in response to physical encounters with other males and/or when the calls of conspecific males exceed some threshold intensity (Brenowitz *et al.* 2001). Aggressive calls may also be emitted in response to physical or acoustic interactions with heterospecifics (Gerhardt 1994).

Males of a number of species have developed considerable plasticity in their acoustic responses (cf Brenowitz *et al.* 2001). Nonetheless, the different signals produced by a male are typically spectrally and/or temporally distinct (Narins *et al.* 2000) and accordingly can be recorded, measured and categorised. We visually examined recording sequences and used conventional statistical analyses to look for consistently distinct acoustic signals with respect to spectral and/or temporal structure.

Recording methods

Calls of fourteen frogs in total were tape-recorded on the 21 August 2000 and 21 September 2000 at Perry Lakes, Perth, Western Australia (31° 56' 40" S, 115° 46' 50" E). Calling males were concentrated along approximately 40 m of shoreline, and the chorus contained about 40 calling males on both occasions. Frogs called from emergent macrophytes and were usually perched between 5 and 60 cm above the water level.

Recordings were made on a Sony Professional Walkman (WM-D6C; frequency response for type I cassette 40 - 15,000 Hz; $\pm 0.3\%$ tape speed variation; manufacturers specifications) with a Beyer Dynamic M88N (C) microphone. Snout-vent length (± 1 mm) was obtained for 11 of the 14 frogs by placing the dorsal surface of the frog flat against a plastic ruler. Dry-bulb air temperature at each call-site (± 0.2 °C) was measured using a Miller & Weber cloacal thermometer directly after the calling sequence of each frog was recorded. Temperatures ranged from 11 to 16 °C ($14.7 \pm \text{sd } 1.9$ °C). Frogs were recorded for a minimum of 3.5 minutes (mean $5.2 \pm \text{sd } 0.45$). Calls were sampled at 44.1 kHz and analyzed using Cool Edit Pro (Syntrillium Software Corporation, Phoenix, v 1.2) running on an IBM microcomputer.

Seven call properties were measured following the procedures of Littlejohn *et al.* (1993) and Gerhardt & Huber (2002). The call properties measured were call duration (ms), pulse number, pulse duration (ms), pulse rate (pulses s^{-1}), low frequency peak (kHz), dominant

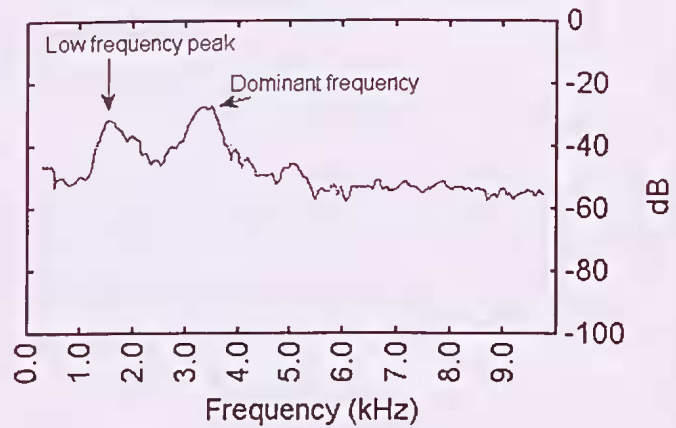


Figure 1. Power spectrum derived from a type 1 call shows the two peaks used to obtain the low frequency peak and dominant frequency.

frequency peak (kHz), and inter-note duration (ms). Pulse rate for call type 2 (see results) is reported as the average of the two notes. Dominant and low-peak frequencies (Fig 1) were measured from a power spectrum of the entire call. Where a significant relationship was detected, call properties were standardized for temperature by converting them to the average call site temperature for the recordings used in this study (14.7 °C). To allow the determination of the direction of relationships, slopes (b_y) are reported with all regression analyses. The first ten males to produce between three and five of each call type were used to investigate the differences between call types. Variation in each call property between call types 1, 2 and 3 were analysed with single-factor ANOVA (Diekhoff 1992). We also performed a discriminant function analysis (DFA; Diekhoff 1992) with the call properties as the dependent variables and three call types as the independent variable. All statistical analyses were conducted with Statistica (v 5.0).

Results

Call types

The number of calls recorded for each frog varied from 4 to 109 (mean 37.5 ± 6.9). The waveforms revealed four distinct calls (Fig 2). Call type 1 was a pulsed call.

Table 1

Descriptive statistics for properties of call types 1, 2 and 3 of *Litoria adelaidensis*. Values are mean \pm sd; sample size is 10 for each call property.

	Call type 1	Call type 2	Call type 3
Call duration (ms)	105.6 \pm 26.5	165.2 \pm 51.0	46.2 \pm 11.5
Pulse number	9.8 \pm 1.6	6.7 \pm 1.8	2.7 \pm 0.41
Pulse duration (ms)	8.1 \pm 0.96	9.7 \pm 1.5	10.3 \pm 3.1
Pulse rate (pulses s^{-1})	86.5 \pm 12.0	66.5 \pm 15.3	41.2 \pm 5.7
Low peak frequency (kHz)	1.8 \pm 0.05	1.8 \pm 0.06	1.8 \pm 0.03
Dominant frequency (kHz)	3.8 \pm 0.14	3.8 \pm 0.16	3.7 \pm 0.12
Inter-note duration (ms)		78.2 \pm 33.9	

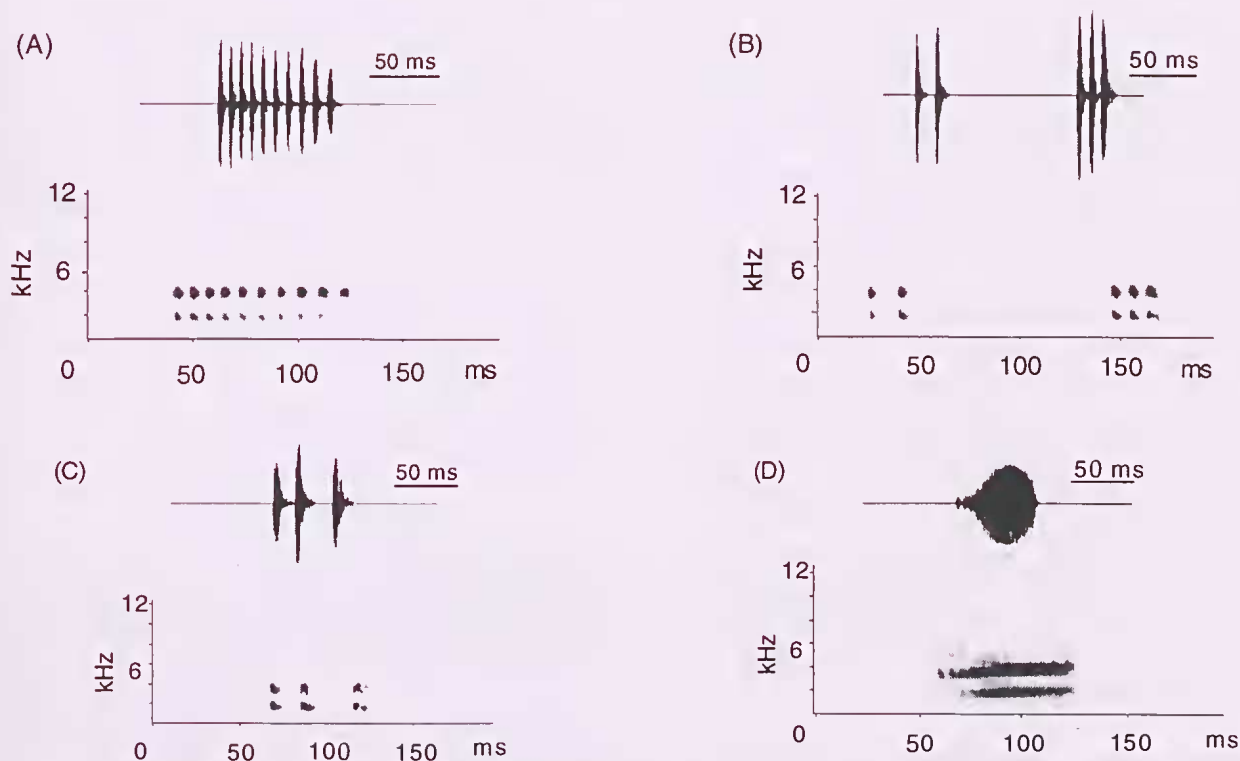


Figure 2. Waveforms (upper) and audiospectrograms (lower) of four types of acoustic signals produced by males of *Litoria adelaidensis*. (A) call type 1, (B) call type 2, (C) call type 3 and (D) call type 4.

Call type 2 was similar to call type 1, but had two pulsed notes with a mean inter-note duration of 78.2 ms (maximum = 136.0 ms). All other calls consisted of a single note only. Call type 3 was a short pulsed call that was similar in duration to one of the notes in call type 2 (Fig 3). Call type 3 was consistently shorter in duration and had fewer pulses (Fig 3) than the other call types. Call type 4 was only recorded from one individual but was often heard in choruses; it was an unpulsed whistle ($n = 3$, call duration 65.3 ms, low frequency peak 1.7 kHz, dominant frequency peak 3.5 kHz). All pulsed call types had two distinct frequency peaks (Figs 1 and 2) with the higher, or dominant, frequency peak the louder of the two (Table 1).

Variation with temperature

A significant amount of the variation in the both frequency peaks in call types 1, 2, and 3 was explained by differences in temperature. For low peak frequencies; call type 1: $r^2 = 0.66$ and $b_y = 0.04$ ($F_{1,8} = 15.6$, $P < 0.01$); call type 2: $r^2 = 0.62$ and $b_y = 0.04$ ($F_{1,8} = 13.1$, $P < 0.01$); and call type 3: $r^2 = 0.85$ and $b_y = 0.04$ ($F_{1,8} = 43.6$, $P < 0.01$). For dominant peak frequencies; call type 1: $r^2 = 0.60$ and $b_y = 0.09$ ($F_{1,8} = 11.9$, $P < 0.01$); call type 2: $r^2 = 0.6$ and $b_y = 0.10$ ($F_{1,8} = 13.9$, $P < 0.01$); and call type 3: $r^2 = 0.77$ and $b_y = 0.10$ ($F_{1,8} = 26.7$, $P < 0.01$).

Variation with body size

A significant amount of variation in dominant frequency was explained by body size for call types 1, 2 and 3. For call type 1: $r^2 = 0.61$ and $b_y = -0.02$ ($F_{1,8} = 12.6$, $P < 0.01$); call type 2: $r^2 = 0.53$ and $b_y = -0.03$ ($F_{1,8} = 9.1$, $P < 0.05$); call type 3: $r^2 = 0.40$ and $b_y = -0.02$ ($F_{1,8} = 5.4$, $P < 0.05$). The low-frequency peak did not vary significantly

with body size for any of the call types (call type 1: $F_{1,8} = 0.80$, $P = 0.40$, call type 2: $F_{1,8} = 1.3$, $P = 0.28$, call type 3: $F_{1,8} = 4.2$, $P = 0.07$).

A significant amount of the variation was explained by variation in body size for call duration and pulse number in call type 1 ($r^2 = 0.46$, $b_y = -6.8$, $F_{1,8} = 6.9$, $P < 0.05$ and $r^2 = 0.57$, $b_y = -0.65$, $F_{1,8} = 10.7$, $P < 0.05$, respectively) and call duration and inter-note duration in call type 2 ($r^2 = 0.55$; $b_y = -16.2$, $F_{1,8} = 9.7$, $P < 0.05$ and $r^2 = 0.47$, $b_y = -9.5$, $F_{1,8} = 7.1$; $P < 0.05$, respectively).

Among call type analyses

The three pulsed-calls varied significantly from each other in call duration ($F_{2,36} = 37.3$, $P < 0.01$), pulse number ($F_{2,36} = 57.8$, $P < 0.01$) and pulse rate ($F_{2,36} = 22.4$, $P < 0.01$, Fig. 3) only. Call type 2 could be differentiated by the presence of two notes. Call types 1 and 3 could be differentiated by their pulse number and duration (Fig 3).

Call type 1 was produced most commonly (mean of the percentage of calls that were call type 1 for each individual = $60.3 \pm 6.6\%$) followed by call type 3 ($24.0 \pm 5.7\%$) and call type 2 ($15.3 \pm 3.7\%$). The individual that produced the type 4 calls did so on three occasions.

Discriminant analysis

The results of DFA confirmed the univariate analyses, showing that call types 1, 2, and 3 differed from each other in call duration, pulse number and pulse rate (Wilk's $\lambda = 0.02$, approx $F_{12,44} = 25.1$, $P < 0.01$; Fig 4). Even though some overlap occurred between call types in call duration, pulse number and pulse rate (Fig 3), the DFA produced clear separation of each call type (Fig 4).

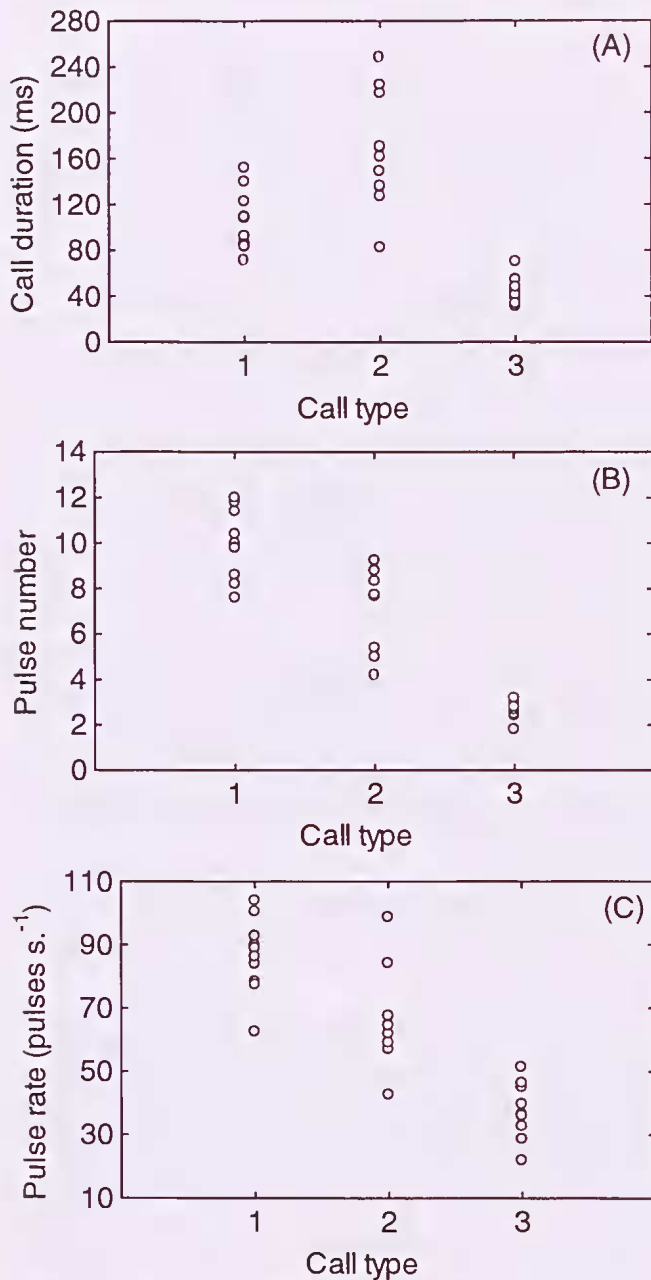


Figure 3. Scatter plots showing differences between the three call types of *Litoria adelaidensis* in (A) call duration, (B) pulse number and (C) pulse rate.

Discussion

The slender treefrog *Litoria adelaidensis* has a repertoire of acoustic signals that includes both pulsed and unpulsed calls. Four call types were recognised; call types 1, 2 and 3 were pulsed and call type 4 was unpulsed. All call types had two main frequency peaks. The presence of two frequency peaks has been shown to be an important factor in female mate choice in other hylid species (e.g. *Hyla versicolor*; Gerhardt & Huber 2002) and consequently may influence female mate choice in *L. adelaidensis*. This awaits further study.

Only dominant frequency varied consistently with male size across all pulsed call types. A negative correlation between dominant frequency and body length is usual in frogs (Gerhardt 1994) and is probably related

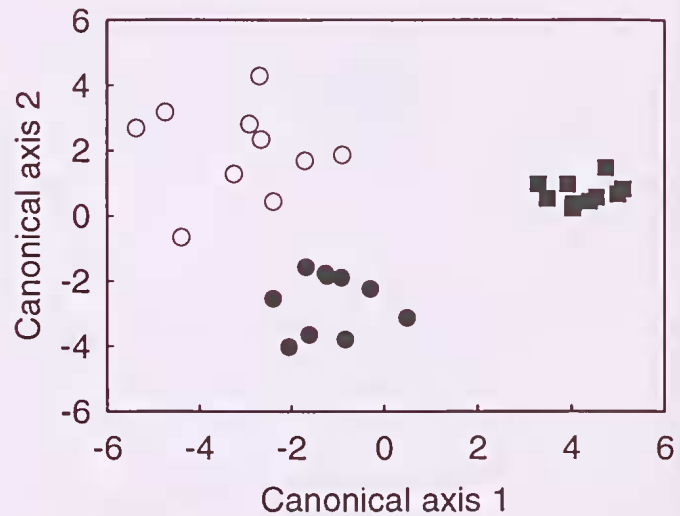


Figure 4. Distribution of the call types (1: solid circle, 2: open circle, and 3: solid square) of male *Litoria adelaidensis* along two canonical discriminant axes derived from a DFA of call properties on call type. The correlations between canonical axis 1 and call duration, pulse number, pulse rate, pulse duration, low frequency peak and dominant frequency were -0.45, -0.50, -0.39, 0.06, -0.10, and -0.11 respectively. The correlations between canonical axis 2 and call duration, pulse number, pulse rate, pulse duration, low frequency peak and dominant frequency were 0.20, -0.67, and -0.53, 0.20, 0.13, and -0.01 respectively.

to the size of the male's vocal chords (McAlister 1961). The durations of call types 1 and 2 also varied inversely and significantly with body size, as larger males produced shorter calls. Accordingly, the relationships between body size and both frequency and call duration suggest that size and possibly age (frogs generally increase in size with age) may influence male calling tactics in this species. However, the relationship between size and frequency does not hold for all species (e.g. Lardner & bin Lakim 2002).

The pulsed calls (call types 1, 2 and 3) differed in temporal structure (in particular, the number of pulses and presence or absence of more than one note). Similar variation in temporal structure of different calls in the repertoire of several species has been reported (e.g. Jehle & Arak 1998; Narins *et al.* 2000; Brenowitz *et al.* 2001). The occurrence of significant variation in the temporal but not spectral structure of the calls of *L. adelaidensis* may indicate different selection regimes and/or differential responses to selection. For example, there may have been stabilizing selection on the frequency components of the calls, but directional selection on the temporal structure (cf Gerhardt 1994).

Call type 1 may function to attract females (cf Littlejohn 1957; Gerhardt 1994) as this call type was produced more often than any other. We have no basis to suggest a function for call types 2 and 3, but the series of pulsed calls described here may represent a graded set of signals. Graded signals have been documented in other species such as *Uperoleia* (Robertson 1984), *Rana* (Jehle & Arak 1998) and *Hyla* (Schwartz 2001) and typically appear to represent an escalation in response to decreasing distance to an opponent or female (Gerhardt & Huber 2002). For example, there is a negative

correlation for *Rana nicobariensis* between duration of its advertisement call and proximity of its nearest calling neighbour (Jehle & Arak 1998). *Litoria adelaidensis* males often call from emergent vegetation (typically reeds), and accordingly the physical structure of their breeding habitat may impose a limitation to male spacing. These circumstances may favour graded interactions between males.

Call type 4 was rarely recorded and may function in male-male interactions. On other occasions, we have observed pairs of males in close proximity, on a single reed stem, making this call. However, the locations of other males when call type 4 was recorded in this study are unknown. These observations, and the rarity of call type 4, indicate that it may act as a short range "encounter" call (e.g. Robertson 1984; Halliday & Tejedo 1995). This conclusion needs confirmation by further observation and playback experimentation.

Studies designed to further investigate the functions of the call types of *L. adelaidensis* are required. These need to examine responses of both males and females to call playback, and account for how the intensity of the calls of neighbours and male size may affect calling tactics (e.g. Robertson 1984; Rose & Brenowitz 1997; Brenowitz & Rose 1999). The slender treefrog may lend itself to studies concerned with the evolution of vocal repertoires and vocal behaviours in frogs.

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